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Cinereomyces wuliangshanensis sp. nov. from China

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Abstract—A new poroid wood-inhabiting fungal species, *Cinereomyces wuliangshanensis,* is proposed based on morphological and molecular characters. The species is characterized by resupinate brittle basidiomata with a white pore surface, a dimitic hyphal system with clamped generative hyphae, and subglobose to broadly ellipsoid hyaline thin-walled smooth basidiospores (4.2–5.1 × 2.9–3.8 µm). Sequences were generated from the internal transcribed spacer (ITS) region of nuclear ribosomal RNA gene and phylogenetically analyzed using maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogeny strongly (100% BS, 100% BT, 1.00 BPP) supports *C. wuliangshanensis* in a monophyletic lineage grouping with *C. lindbladii* and a clade comprising *Obba rivulosa* and *O. valdiviana.*

Key words—*Gelatoporiaceae*, *Polyporales,* taxonomy, wood-inhabiting fungi, Yunnan Province

Introduction

Cinereomyces Jülich (*Gelatoporiaceae*, *Polyporales*) is characterized by resupinate poroid basidiomata with a white to cream to greyish pore surface with encrustations in trama or tube mouths, a dimitic hyphal system with clamp connections on generative hyphae, skeletal hyphae that dissolve in KOH,

cystidia absent, and basidiospores that are cylindrical to allantoid, hyaline, thin-walled, smooth, non-dextrinoid, and acyanophilous (Jülich 1982, Miettinen & Rajchenberg 2012, Ryvarden & Melo 2014). The genus, which is typified by n *C. lindbladii* (Berk.) Jülich, contains two accepted species (Jülich 1982, Miettinen 2012).

Phylogenetically, Binder & al. (2005) distributed resupinate forms across the major clades of mushroom-forming fungi and nested *Cinereomyces lindbladii* in the core polyporoid clade, grouped with *Dentocorticium sulphurellum* (Peck) M.J. Larsen & Gilb. Nuclear and mitochondrial ribosomal DNA sequences used by Tomšovský & al. (2010) grouped *C. lindbladii* with *Gelatoporia subvermispora* (Pilát) Niemelä and *Obba rivulosa* (Berk. & M.A. Curtis) Miettinen & Rajchenb. Analysis of the taxonomic relationships of four genera—*Cinereomyces*, *Gelatoporia* Niemelä, *Obba* Miettinen & Rajchenb., *Sebipora* Miettinen—nested *C. lindbladii* in the *Cinereomyces* clade, grouped with *G. subvermispora*, *O. rivulosa*, and *S. aquosa* Miettinen. Miettinen & Rajchenberg (2012) grouped *C. lindbladii* with *G. subvermispora* and *Skeletocutis amorpha* (Fr.) Kotl. & Pouzar based on their molecular analysis of *Hymenochaetales* with poroid and hydnoid hymenophores. Further molecular analyses of *Polyporales* by Binder & al. (2013) nested *C. lindbladii* within the *Gelatoporia* clade, grouping *Cinereomyces* with *Gelatoporia*, *Obba*, and *Sebipora*. A revised family-level classification of the *Polyporales* based on nrLSU, nrITS, and RPB1 genes confirmed that *Cinereomyces* belongs in *Gelatoporiaceae* Miettinen & al. (Justo & al. 2017).

During investigations on wood-inhabiting fungi in southern China, an additional taxon was found that could not be assigned to any described species. After examining the morphology and generating a phylogeny from internal transcribed spacer (ITS) region sequences, we propose a new *Cinereomyces* species.

Materials & methods

The specimens studied are deposited at the herbarium of Southwest Forestry University, Kunming, Yunnan Province, P.R. China (SWFC). Macromorphological descriptions were based on field notes. Colour terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens and observed under a light microscope. Abbreviations used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both non-amyloid and non-dextrinoid, L = mean spore length (arithmetic average of all basidiospores), W = mean spore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios, |n (a/b) = (a) number of basidiospores measured from (b) number of specimens.

Table 1. Species, specimens, and sequences used in this study (new sequences in **bold**).

Species name	Sample no.	GenBank no.	Reference
		ITS	
Cinereomyces lindbladii	Kotiranta 19911	FN907909	Miettinen & Rajchenberg 2012
	FBCC 117	HQ659223	Miettinen & Rajchenberg 2012
	KH Larsson 12078	FN907906	Miettinen & Rajchenberg 2012
C. wuliangshanensis	**CLZhao 3405 [T]**	MT664802	Present study
	CLZhao 3409	MT664803	Present study
Gelatoporia subvermispora	Dai 3120	HQ659226	Miettinen & Rajchenberg 2012
	Miettinen 9079	HQ659229	Miettinen & Rajchenberg 2012
	Niemela 5978	HQ659227	Miettinen & Rajchenberg 2012
	BRNU 592909	FJ496694	Miettinen & Rajchenberg 2012
	Spirin 2156	HQ659228	Miettinen & Rajchenberg 2012
	Kinnunen 1052	HQ659225	Miettinen & Rajchenberg 2012
Lopharia cinerascens	EL6397	AY463440	Miettinen & Rajchenberg 2012
Obba rivulosa	Miettinen 8054	HQ659231	Miettinen & Rajchenberg 2012
	FBCC 938	HQ659233	Miettinen & Rajchenberg 2012
	Kotiranta 16702	HQ659232	Miettinen & Rajchenberg 2012
	Penttila 14135	HQ659234	Miettinen & Rajchenberg 2012
	Penttila 14441	FJ496691	Miettinen & Rajchenberg 2012
	Penttila 15077	HQ659230	Miettinen & Rajchenberg 2012
O. valdiviana	Gates FF503	HQ659235	Miettinen & Rajchenberg 2012
	CIEFAP 336	HQ659236	Miettinen & Rajchenberg 2012
Sebipora aquosa	Miettinen 8680	HQ659240	Miettinen & Rajchenberg 2012
	Miettinen 12032	HQ659241	Miettinen & Rajchenberg 2012
	Miettinen 8868	HQ659242	Miettinen & Rajchenberg 2012
	Miettinen 9265	HQ659243	Miettinen & Rajchenberg 2012

Genomic DNA was obtained from dried specimens using a CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd) following the manufacturer's instructions slightly modified by grinding a small (~30 mg) dried fungal sample to powder with liquid nitrogen; the powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min; thereafter 0.4 mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously; after centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with

0.45 mL binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 100 mL elution buffer was added to the middle of adsorbed film to elute the genomic DNA. The ITS region was amplified with primer pair ITS5 and ITS4 (White & al. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "G-INS-i" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 26522). Sequence of *Lopharia cinerascens* (Schwein.) G. Cunn. obtained from GenBank was used as an outgroup to root tree following Miettinen & Larsson (2011) in the ITS analysis.

Maximum parsimony analysis was performed using ITS sequences dataset. Approaches to phylogenetic analysis followed Zhao & Wu (2017) and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT). Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller & al. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 1 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (BS) > 70%, maximum parsimony bootstrap (BT) >50%, or Bayesian posterior probabilities (BPP) >0.95.

Phylogenetic results

The ITS dataset (Table 1) included sequences from 24 fungal specimens representing seven taxa. The dataset had an aligned length of 570 characters, of which 417 characters were constant, 26 parsimony-uninformative, and 127 parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 213, CI = 0.869, HI = 0.151, RI = 0.959, RC = 0.832). The best-fit model for ITS alignment estimated and applied in the Bayesian was lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian resulted in a similar topology with an average standard deviation of split frequencies = 0.009931 (BI).

The ITS phylogeny (Fig. 1) supported *Cinereomyces wuliangshanensis* as a new taxon in *Cinereomyces,* grouped with *C. lindbladii* and a clade comprising *Obba rivulosa* and *O. valdiviana* (Rajchenb.) Miettinen & Rajchenb.

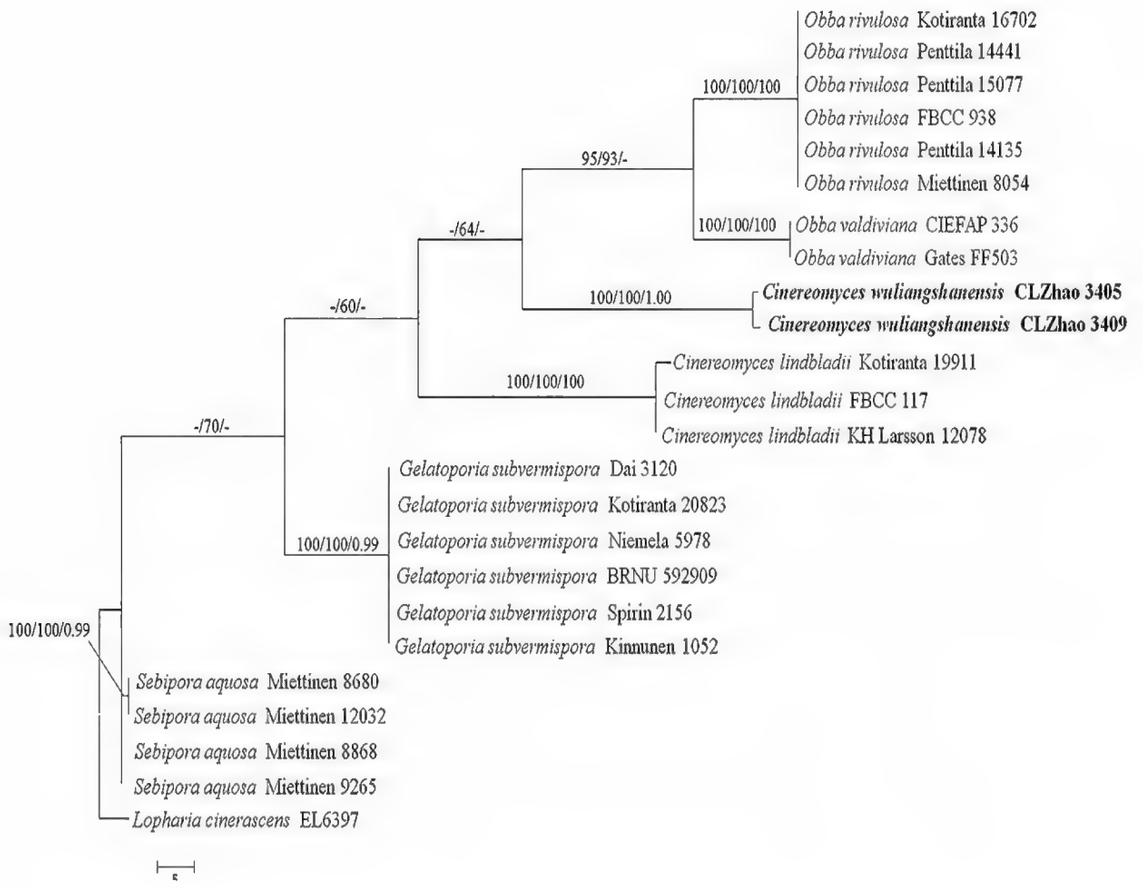

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Cinereomyces wuliangshanensis* and related species based on ITS sequence analyses. Branches are labeled with maximum likelihood bootstrap >70%, parsimony bootstrap proportions >50%, and Bayesian posterior probabilities >0.95.

Taxonomy

Cinereomyces wuliangshanensis C.L. Zhao & K.Y. Luo, **sp. nov.** Figs 2, 3

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Differs from *C. lindbladii* by its white to cream to olivaceous buff pore surface and smaller basidiospores.

Type: Yunnan Province, Puer, Zhenyuan County, Wuliangshan National Nature Reserve, Xieqipo Park, on angiosperm trunk, 1 October 2017, CLZhao 3405 (**Holotype**, SWFC 003405; GenBank MT664802).

Etymology: The specific epithet *wuliangshanensis* (Lat.) refers to the collection locality (Wuliangshan) of the type specimen.

Basidiomata annual, resupinate, soft, without odor or taste when fresh, becoming brittle upon drying, ≤9 cm long, 4.5 cm wide, and 1.5 mm thick at centre. Pore surface white when fresh, white to cream to olivaceous buff upon drying; pores round to elongated, 5–6 per mm; dissepiments thin, entire. Sterile margin narrow, white, ≤5 mm wide. Subiculum white, thin, ≤0.5 mm thick. Tubes white, corky, ≤1 mm long.

Hyphal structure dimitic; generative hyphae with clamp connections, skeletal hyphae IKI–, CB–; tissues swelling in KOH.

Subiculum generative hyphae hyaline, thin-walled, unbranched, 1.5–2.2 µm in diam.; skeletal hyphae rare, hyaline, thick-walled with a wide lumen, frequently branched, interwoven, 4–5 µm in diam.

Tubes generative hyphae hyaline, thin-walled, unbranched, 1–1.7 µm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, frequently branched, interwoven, 3.5–4 µm in diam. Cystidia and cystidioles absent; basidia 15.2–16.1 × 5.1–5.3 µm, barrel-shaped, with four sterigmata and a basal clamp connection; basidioles dominant, mostly barrel-shaped, but slightly smaller than basidia.

Basidiospores (n = 60/2), (3.7–)4.2–5.1(–6.2) × (2.6–)2.9–3.8(–4.4) µm, L = 4.81 µm, W = 3.39 µm, Q = 1.40–1.43, subglobose to broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–.

Type of rot: white.

Additional specimen examined: **CHINA. Yunnan province. Puer:** Zhenyuan County, Wuliangshan National Nature Reserve, Xieqipo Park, on angiosperm trunk, 1 October 2017, CLZhao 3409 (SWFC 003409; GenBank MT664803).

Discussion

The current study proposes and describes a new species, *Cinereomyces wuliangshanensis,* based on phylogenetic and morphological analyses.

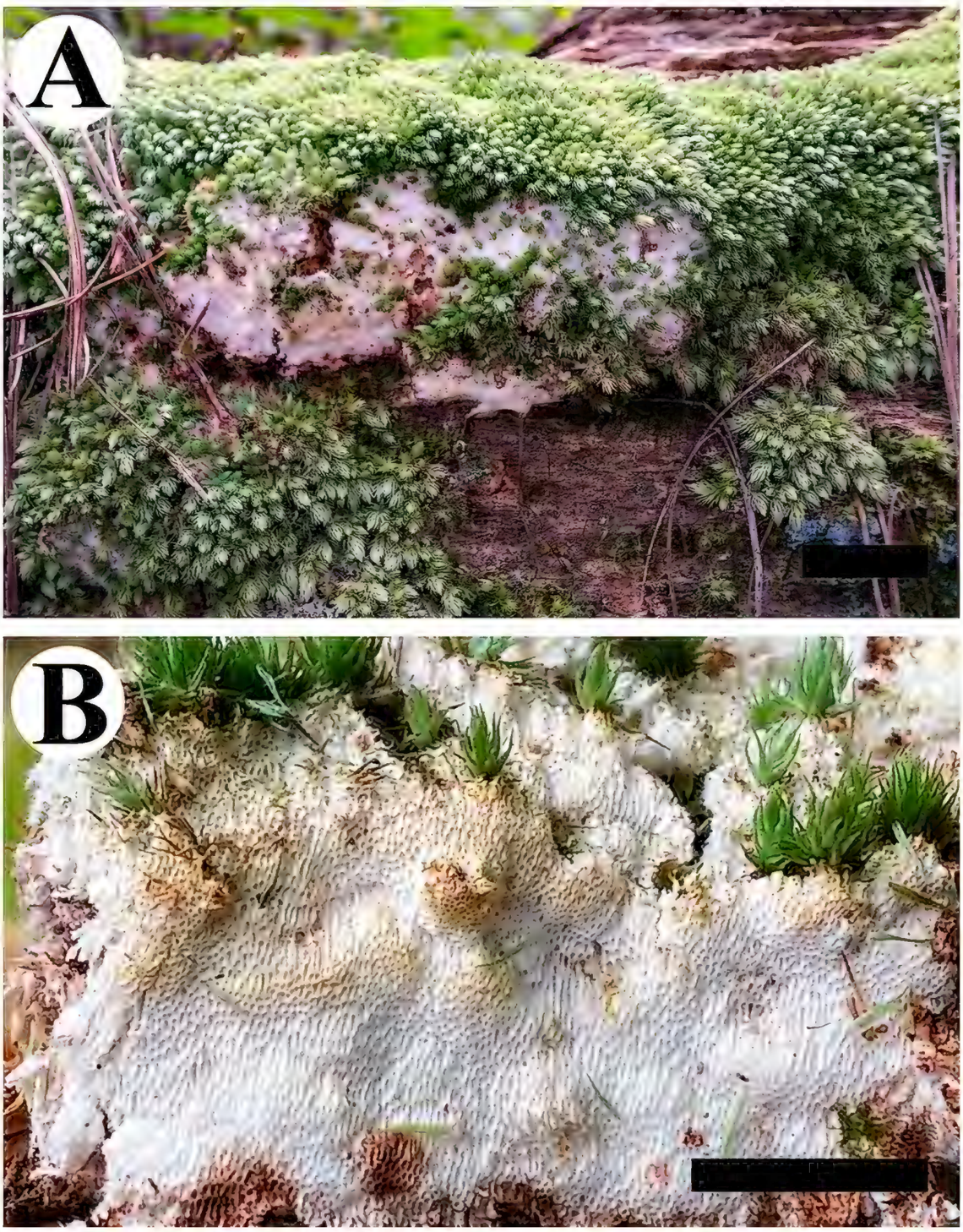

FIG. 2. *Cinereomyces wuliangshanensis* (holotype, SWFC 003405). Basidiomata. Scale bars: A = 2 cm; B = 0.5 mm.

The phylogenetic analysis of the nrDNA ITS region by Miettinen & Rajchenberg (2012) grouped *Cinereomyces* closely with three related genera—*Obba, Sebipora*, and *Gelatoporia*. Our current phylogeny strongly (100% BS,

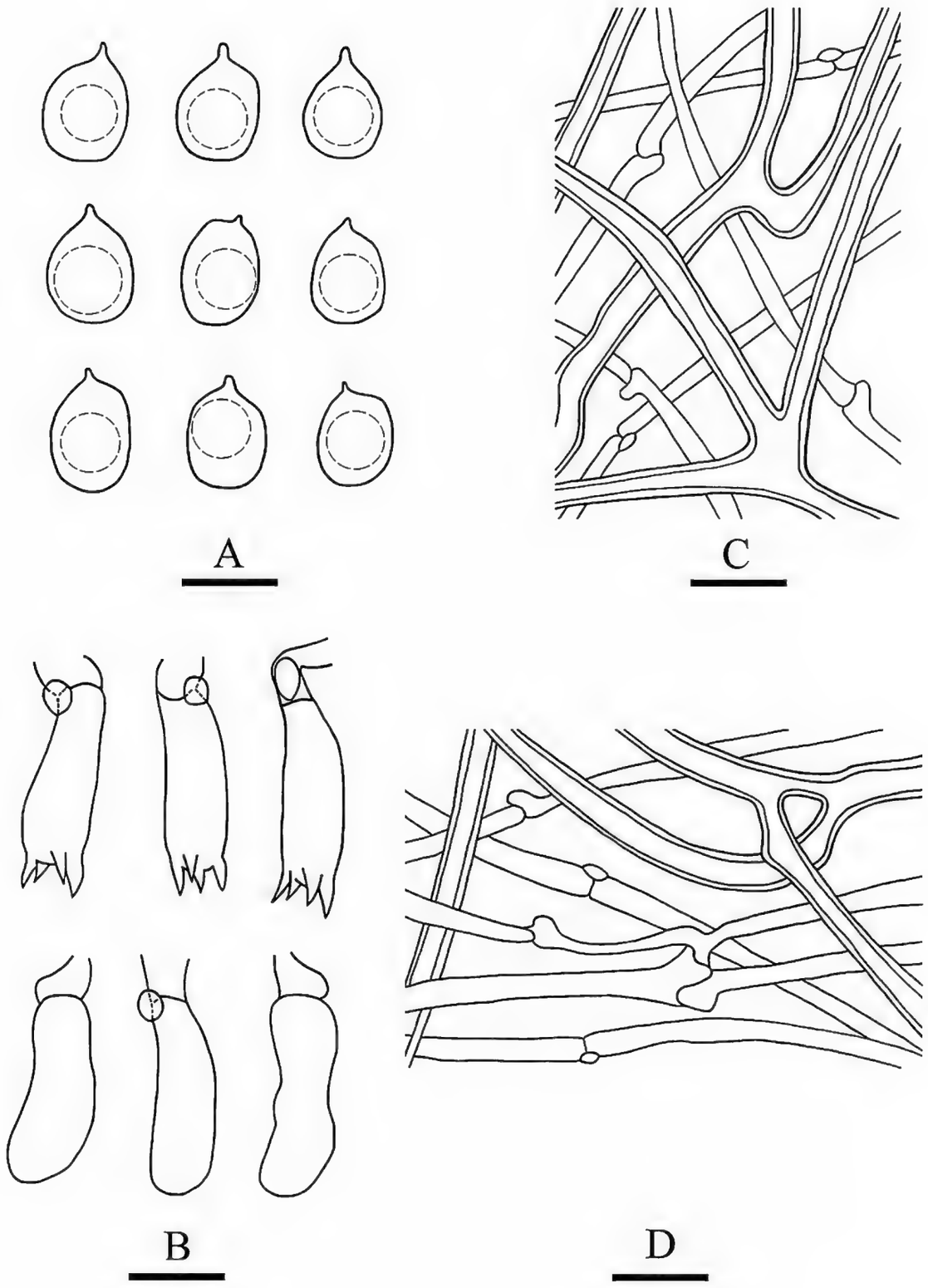

Fig. 3. *Cinereomyces wuliangshanensis* (holotype, SWFC 003405).
A. Basidiospores; B. Basidia and basidioles; C. Hyphae from trama;
D. Hyphae from subiculum. Scale bars: A = 5 *μm;* B–D = 10 μm.

100% BT, 1 BPP) supports the new taxon in a monophyletic lineage that groups with *C. lindbladii* and a clade comprising two species, *Obba rivulosa* and *O. valdiviana*.

Morphologically, *C. lindbladii* can be distinguished from *C. wuliangshanensis* by its greyish pore surface and larger basidiospores (5–7 × 1.5–2 µm; Ryvarden & Melo 2014); *O. rivulosa* differs from *C. wuliangshanensis* by a monomitic hyphal system and thin to slightly thick-walled and wider basidiospores (4.6–5.2 × 3.7–4.3; Miettinen & Rajchenberg 2012); *O. valdiviana,* also distinguished by its monomitic hyphal system, is further distinguished by its cyanophilous and wider basidiospores (4.6–5.3 × 4.2–5.0; Miettinen & Rajchenberg 2012).

Cinereomyces dilutabilis (Log.-Leite & J.E. Wright) Miettinen shares a dimitic hyphal system with *C. wuliangshanensis*. However, *C. dilutabilis* is distinguished by its light brownish basidiomata with smaller (6–8 per mm) pores and narrower basidiospores (4.8–5.5 × 2.4–2.8; Miettinen 2012).

With respect to distribution, *Cinereomyces* species are a rarely studied group of *Basidiomycota* (Loguercio-Leite & Wright 1998, Núñez & Ryvarden 2001, Dai 2012, Ryvarden & Melo 2014). Previously, only one species of *Cinereomyces*, *C. lindbladii,* was recorded in China (Dai 2012). However, the diversity of *Cinereomyces* species in China is still not well known, especially in southern China. In addition to our new species, *C. wuliangshanensis,* other related *Cinereomyces* taxa have been described from this region (Zhao & Cui 2012, Liu & al. 2017, Zhao & al. 2017, Zhao & Ma 2019). There is still a lot of room to explore the diversity of *Cinereomyces* species in southern China.

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